Running SDS-PAGE gels

Introduction

For SDS-PAGE analysis and Western blot

Materials

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  - 5x SDS_PAGE sample buffer
  - samples
  - gel

Procedure

1. Before loading, add 1 volume 5x SDS-PAGE sample buffer to 4 volumes of protein sample (i.e., add 2 µl sample buffer to 8 µl sample giving a final volume of 10 µl, also 5ul is enough). Vortex briefly and heat at 95°C for 5 min. **Tip**: During heating at 95°C, release pressure build up in tubes by briefly opening lids, or piercing a small hole in the lid with a needle. After heating, samples should be briefly centrifuged and vortexed.

2. Load samples and run gel. For electrophoresis conditions refer to the recommendations provided by the manufacturer of the apparatus. **Tip**: Before loading the samples, rinse out wells with 1x electrophoresis buffer using a suitable syringe and needle. **Tip**: Load empty wells with 1x SDS-PAGE sample buffer to ensure that sample lanes do not spread out. **Tip**: Ensure that the electrodes are correctly connected. The proteins will migrate towards the positive (labeled +, usually red) electrode.

3. Run the gel until the bromophenol blue dye reaches the bottom edge. This usually gives a satisfactory spread of protein bands.